REMARKS

The following comments address the issues raised in the Office Action in the order in which they appear. Favorable reconsideration is respectfully requested.

Drawings:

Applicant acknowledges the drawing requirements noted on the Form PTO-948 that accompanied the Office Action. The only informality noted by the Draftsman is a margin that is too narrow in Figs. 1 and 3. A full set of formal drawings, complying with the margin requirements, will be submitted upon an indication of allowable subject matter.

Rejection Under 35 USC §112, First Paragraph:

Applicants respectfully traverse this rejection on both procedural and substantive grounds.

Procedurally, this rejection is traversed because the Office Action fails to identify which claims have been made subject to the rejection. Applicant is therefore unable to formulate a complete reply to the Examiner's comments. For purposes of the remarks that follow, Applicant assumes that all of claims 1-29 have been made subject to this rejection.

This application is further traversed on procedural grounds because the Office cites in support of this rejection U.S. Patent No. 5,200,313, to *Carrico*. The *Carrico* patent, however, is not of record in this application. A copy of the *Carrico* patent was not provided to Applicant by the Office, nor has the *Carrico* patent been formally cited as prior art that was considered by the Office during its examination of the subject claims. The *Carrico* patent is not cited in the application itself, and it was not submitted to the Office in Applicant's earlier-submitted Information Disclosure Statement and Form 1449. It is therefore respectfully submitted that until the *Carrico* patent is made officially of record in this application, the Office's reliance on the *Carrico* patent is improper.

From a substantive viewpoint, Applicant respectfully submits that Office has not properly presented a *prima facie* finding of lack of enablement. The Office has the burden of providing sound scientific reasons, supported by the record, why the specification fails to

properly enable the claims. (See, for instance, *In re Angstadt*, 190 USPQ 214 (CCPA 1976).) As part of that burden, the Office must present evidence showing that the disclosure requires undue experimentation. (*Id.* at 219.) The key concept of non-enablement is "undue," not "experimentation." In short, satisfaction of the enablement requirement of §112 is not voided by the necessity for some experimentation, such as routine screening. (*Id.*) A considerable amount of experimentation is permissible if it is routine or if the specification provides a reasonable amount of guidance with respect to how the experiments should proceed. (See also *In re Jackson*, 217 USPQ 804 (Bd. App. 1982).)

This rejection is traversed because all of the technical considerations recited in the Office Action are routine, inescapable aspects of nucleic acid hybridization and amplification. These concerns are well know to one of skill in the art, and thus do not need to be discussed in great detail in the application. (The Office encourages Applicants to omit from the specification those items of technical detail that are well known to researchers skilled in the field.) Hence, although the requirements for successful PCR amplification are many, they are simply routine requirements that are addressed every single day in laboratories around the world. These considerations do not constitute undue experimentation, but routine optimization of a notoriously finicky reaction, the PCR.

In fact, the very existence of the *Carrico* patent, cited by the Office in support of this rejection, undercuts the Office's argument that these consideration constitute undue experimentation. The teaching of the *Carrico* patent is readily available to one of skill in the art - hence, its teaching would be part of the background knowledge presumed to be understood from the outset by one attempting to practice the invention as claimed. The large list of concerns contained at pages 3 and 4 of the Office Action are addressed in painstaking detail in prior art literature such as the ubiquitous and extensively published *Sambrook, Fritsch, & Maniatis* laboratory manuals entitled "Molecular Cloning." Hence, one of skill in the art would know that if amplification did not occur, perhaps the DNA should be re-precipitated to remove impurities. Testing for GC content (in order to determine optimum cycling conditions) is well known and routine when amplifying unknown DNA sample. "Specificity" is a term that is only loosely defined in the Office Action. If a primer binds with perfect complementarity, that primer can be considered monospecific, regardless of how many

times the sequence appears in the target. The fact that the amplified region of DNA might be different is a different aspect of "specificity." In the present invention, however, the goal is to amplify ALL of the regions that are bounded by a region of a given sequence. In this circumstance, "specificity" boils down to "stringency." "Stingency" however, is also a well known aspect of hybridization. If the goal is to avoid mismatches between the primer and the target, one of skill in the art knows to increase the stringency of the amplification protocol. Concentration of the reagents, incubation time, the presence of denaturation agents, volume exclusion agents, etc. are all routine considerations in the art. That is why PCR reactions are virtually never run as a single reaction. They are routinely run in many duplicate runs, to ensure that at least one of the runs will yield suitable results.

Regarding the source of the DNA, DNA is DNA. The PCR functions in exactly the same fashion regardless of the source of the template DNA. Be it plant, animal, eukaryote, or prokaryote, DNA subjected to the PCR reacts in the same fashion. Hence, the Office's reliance on *In re Fischer* attempts to impugn the functionality of the PCR itself. One of skill in the art need not be instructed on how to perform a reaction that is performed perhaps millions of times daily, around the globe. The PCR is not impossible, it is just finicky and requires a deft hand and a good deal of patience. Again, however, the finicky nature of the PCR is because it is a fundamentally empirical reaction. The ideal conditions for ANY PCR reaction simply cannot be dictated *a priori*. The ideal conditions are worked out through repetitive, laborious, but routine, optimization.

Regarding "specificity," any reference to "specific" amplification has been removed from the claims. Hence, this aspect of the rejection has been rendered moot. Nevertheless, to make the record complete, the Office Action does not provide any objective evidence on how or even whether any such non-specific amplification would make the claimed method inoperable. The method might work perfectly well in spite of any non-specific amplification products. In the absence of any objective scientific evidence presented by the Examiner with respect to inoperability, a *prima facie* showing of non-enablement has not been shown.

Further still, even if non-specific sections of DNA were amplified while practicing the present method, that is a possibility in every PCR experiment. As noted above, a *prima facie* showing of non-enablement must be supported by evidence of undue experimentation. The

empirical and often unpredictable nature of the PCR is notoriously well known. Adjustment of primer concentrations, stringency, cycling conditions, etc, is part of the routine nature of the PCR. Modifying these parameters to suit a given application is not undue experimentation at all, but is an unavoidable and routine aspect of the everyday practice of the PCR.

Regarding working examples, the Office Action states that the specification does not contain any working examples. This, while true, is irrelevant to the determination of whether the specification passes muster under §112, first paragraph. There is no requirement under §112 for any working examples to support the claims. *In re Robbins*, 166 USPQ 552 (CCPA 1970), cited with approval by the Court of Appeals for the Federal Circuit in *Amgen v. Chugai*, 18 USPQ2d 1016 (Fed. Cir. 1991).

The Office Action also states that the specification makes reference to prior art methods, yet the specification does not teach how these methods are to be modified for use in the present invention. The prior art methods need not be modified or adapted in any way to practice the claimed invention. The PCR reactions are assembled as described in the specification and run in standard fashion. Nothing more is needed to practice the invention as broadly as it is claimed.

The Office has asked for additional evidence of the operability of the present invention. Applicant therefore submits for the Examiner's consideration the attached Rule 132 Declaration of inventor Periannan Senapathy. The Declaration, which was prepared earlier in response to an Office Action in another of Applicant's patent applications, is admittedly not exactly on point, but provides ample evidence that the description contained in the subject patent application enables the claims as they are presently worded.

Paragraph 2 of his Declaration specifically states that Dr. Senapathy either conducted the experiments contained in his Declaration personally, or that they were carried out under his direction and supervision.

Paragraph 3 of the Declaration presents a brief overview of how an appropriate annealing temperature for a PCR run is selected based upon the average Tm of the two primers. Higher temperatures are more stringent, meaning the higher the temperature of annealing, the more specific the PCR run generally is. Dr. Senapathy also notes in paragraph

3 that the PCR methodology is, by its very nature, empirical, and must be adjusted for each new reaction.

At paragraph 5 of his Declaration, Dr. Senapathy introduces an experiment to determine the primer concentration necessary to amplify specifically a target DNA using a plurality of primers having a fixed portion and a randomized portion.

At paragraph 6, the experimental set-up is described. The template DNA is plasmid DNA (pGEM) and two control primers are described. A plurality of primers having 6 positions of fixed sequence and 8 positions randomized was then constructed. A PCR reaction using this plurality of primers was then conducted at 60°C (a very standard temperature) at various concentrations. The results are discussed in paragraph 7 and Figure 1 of the Declaration. The legend to Figure 1 of the Declaration recites all of the cycling parameters used in this reaction. As noted in paragraph 7, this reaction showed that the ideal concentration for the plurality of primers was 10X.

Paragraph 8 of the Declaration introduces a new experiment in which the number of fixed nucleotides within the plurality of primers was varied and using a much longer piece of template DNA (*E. coli* genomic DNA) (see paragraph 9). As noted in paragraph 9, three separate regions from the E. coli genome were chosen for amplification. Five primers for each of the three regions were constructed: 1) a first, fixed primer; 2) a second, fixed primer; 3) a plurality of partly-fixed second primers with 8 nucleotides fixed and 8 nucleotides fully randomized; 4) a plurality of partly-fixed second primers with 6 nucleotides fixed and 10 nucleotides fully randomized; and 5) a plurality of partly-fixed second primers with 5 nucleotides fixed and 11 nucleotides fully randomized (see paragraph 10).

Paragraph 11 of Dr. Senapathy's Declaration describes the concentration ranges tested, while paragraph 12 presents the exact nature of the five different primer sets for each of the three regions to be amplified specifically.

The purpose of this experiment is to demonstrate that the claimed process works using convention temperatures of annealing. As noted in paragraph 13 of the Declaration, the temperature of reannealing was raised gradually within the normal range used in the PCR protocol. The first experiment was conducted at an annealing temperature (Tm) within the normal range for all the three sets of experiments, namely 60° C. This temperature, however,

is fairly stringent for the experiment with Region 3 (where the average Tm = 61°C), but not so stringent for Regions 1 and 2 (where the average Tm = 65.5° C and 62.2° C, respectively). The DNA from each of the three regions were PCR amplified in separate experiments with whole *E. coli* DNA as template. For each region, the PCR amplification between the first fixed primer and the second fixed primer was tested as a positive control.

The results are discussed in paragraph 14 of Dr. Senapathy's Declaration and in the attached Figure 2. Note that the particulars of the PCR runs are included in the legend to Figure 2 of the Declaration. In short, the results show that the expected band appears in the gel, with no significant non-specific amplification products. Paragraph 14, also notes, as is stated in the specification, that even if bands other than the expected band occur in the PCR products, sequencing would be done using the first primer, which is specific only to the particular DNA band amplified from the first primer location in the target DNA. Thus, the sequencing reaction should produce only the sequence of the specific expected DNA fragment.

This is, in fact, the case, as demonstrated by the sequencing reaction presented in paragraphs 16-18 of Dr. Senapathy's Declaration and the sequencing gels shown in Figure 3 of the Declaration. Most convincing from this experiment is that the expected sequence was obtained using all three different pluralities of partially-fixed, partially-random primers. All three pluralities yielded the same sequence data as the positive control. Clearly, this reaction convincingly demonstrates that the process as described and claimed, can, in fact, yield highly accurate, precise, reproducible, and useful sequencing data. Paragraph 18 of the Declaration specifically states that these results show that the presence of non-specific amplification products in the PCR products does not affect sequencing of the specifically-amplified fragment from the target region.

Beginning at paragraph 20 of his Declaration, Dr. Senapathy introduces another set of experiments designed to demonstrate the effect of primer concentration on the amplification and sequencing reactions. In paragraph 21, the Declaration states that the experiment was conducted with the PCR amplifications carried out with and without increasing the concentration of the randomized second primer, using a DNA template of 12.5 KB. The object of this experiment is to show that increasing the concentration of the primers results in an increased amount of the specific amplification products. The results, discussed in

paragraphs 21 and 22 and inFigures 6A and 6B, show that the primer with 6 random nucleotides at the 5' end (10-fold excess) and the primer with 8 random nucleotides at the 5' end (120-fold excess) amplified the target DNA fragment. The primer with 10 random nucleotides at a 250-fold excess, however, did not amplify the product, indicating that a higher concentration of the primer was needed in this reaction. Dr. Senapathy notes that these results demonstrate that target-specific amplification can be obtained by increasing the concentration of the plurality of partially-fixed, partially-randomized primers.

The sequencing experiment presented at paragraphs 23-25, and the sequencing gels shown in Figures 5A-5D of Dr. Senapathy's Declaration show that the amplification products produced by the reaction are sufficiently pure to be sequenced. Here, as noted in paragraph 25, a series of sequencing runs were performed using various pluralities of primer sets, including sets with 2, 4, 6, and 8, randomized base pairs. The specific primer sets are described in paragraph 25 of the Declaration and the results are depicted, quite convincingly, in the sequencer tracings shown in Figures 5A-5D.

The Declaration concludes with a final experiment, in paragraph 26, showing that the strength of the sequencing signals is inversely proportional to the number of random base pairs in the primer plurality. Here, in a fashion similar to the reaction described immediately above, a series of sequencing runs was performed with primer pluralities having 2, 4, 6, 8, and 10 randomized base pairs. The results show that the peak intensity was reduced as the number of randomized base pairs was increased. Nevertheless, even an 8-fixed/8 random primer was able to generate the correct sequencing data.

Clearly, in light of the objective scientific data presented in Dr. Senapathy's Declaration, data which was generated using the information specifically provided in the specification of the subject application, the specification enables the invention as claimed, in full satisfaction of 35 USC §112, first paragraph. It is therefore respectfully requested that this rejection now be withdrawn.

Rejection of Claims 1-29 Under 35 USC §112, Second Paragraph:

This rejection is believed to have been rendered moot, in part, by appropriate amendment to the claims, and is, in part, respectfully traversed.

This rejection is believed to have been rendered partially moot by removing the word "specifically" from the claims. For sake of clarity, however, the Examiner's question regarding how one is to perform "specific amplification" when a plurality of primers are used and the primers themselves have a random region, is not well taken. "Specificity" in the context of DNA amplification, refers to the fidelity of the base pair match between a primer and its binding site in a template. On one hand, a primer is highly specific (i.e. monospecific) if there are no base pair mismatches between the primer and the location to which it binds. On the other hand, a primer is less specific if it will bind to non-complementary sites on the template (and hence, prime the amplification of regions of DNA not necessarily of interest).

In the context of the present invention, a great many of the plurality of primers used to amplify the template may not have a specific match within the template being probed. Hence, these individual primers will not result in the amplification of any of the template DNA. One or more of the individual primers within the plurality will, however, encounter a perfect complementary base pair match in the template being probed and will hybridize (specifically) to that site and to no other. Amplification from these primers will therefore result in the specific amplification of a nucleic acid fragment flanked by two primers whose base pair sequence is exactly complementary (or nearly exactly complementary) to the site on the template where they hybridized. In short, even though there are more than one set of primer pairs, and even though each primer has a semi-random construction, each individual primer is capable of specifically priming the amplification of a portion of the template.

Applicant traverses this rejection in part because it is not certain where in the claims it is recited that a "region of 'fixed nucleotide sequence' can contain a segment comprised of randomly selected nucleotides." (See Office Action, page 7, last paragraph.) Claims 1 and 19 recite that the region of fixed nucleotide sequence is "arbitrary," not random. See Claim 1, clause (b) and Claim 19, clause (b).

The fixed region of the primer has a base pair sequence that is fixed and unchanging. The actual sequence chosen for the fixed region, however, is arbitrary; the base pair sequence of the fixed region can be selected by the user for whatever reason. Hence, the base pair sequence of the fixed portion of the primers as recited in Claim 1, clause (b) is selected "arbitrarily" by the user. As used here (and as commonly defined), "arbitrary" means

"depending on individual discretion" (Merriam Webster's Collegiate Dictionary, Tenth Ed.). Hence, it is respectfully submitted that the claims are clear on their face. As recited in Claims 1 and 19, each primer within the plurality of primers has a region that is of fixed sequence; that is, every single primer in the plurality has the same fixed region. The nucleotide within this fixed region do not vary from primer to primer within the plurality. The region is fixed.

In light of the above remarks, it is respectfully submitted that the rejection of Claims 1-29 under 35 USC §112, second paragraph, has been overcome. Withdrawal of the rejection is respectfully requested.

Conclusion:

Applicant respectfully submits that the application is now in condition for allowance. Early notification of such action is earnestly solicited.

Respectfully submitted,

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